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Tumor inflammatory response induced by immunization with autologous melanoma cells conjugated to dinitrophenol(DNP). D. Bard, M.J. Mastrangelo, C. Green, C. Clark, and E. Hart. Thomas Jefferson University, Philadelphia, PA 19107.

Treatment of melanoma patients with an autologous vaccine preceded by low dose cyclophosphamide (CY) induces delayed-type hypersensitivity (DTH) to melanoma cells, and in some cases, regression of metastatic tumors. Now, we are attempting to increase the efficiency of the process by immunizing with tumor cells conjugated to the hapten, DNP. Patients with metastatic melanoma were sensitized to DNP by topical application of dinitrochlorobenzene (DNCB). Two weeks later, they were injected with a vaccine consisting of 10-25x10(6) autologous, irradiated melanoma cells conjugated to DNP and mixed with BCG. CY 300 mg/M^2 IV was given 3 days before DNCB or vaccine. Of 4 patients evaluable so far, 3 have developed a striking inflammatory response in tumor masses after 2 vaccine treatments (8 weeks). Patient #1 developed erythema and swelling in the >50 large (1-3 cm) dermal metastases on her leg and lower abdomen, followed by ulceration and drainage of necrotic material, and some are beginning to regress. Biopsy showed infiltration with CD4+ and CD8+ T lymphocytes. Patient #2 developed erythema and swelling in the skin of her lower abdomen and groin overlying large (8 cm) nodal masses. These have not yet regressed, but have changed in consistency from rock-hard to fluctuant. Patient #3 exhibited moderate erythema in the skin overlying subcutaneous metastases. All 3 patients have developed DTH to both DNCB and to DNP-conjugated autologous lymphocytes. Although these results are preliminary, they suggest that this new strategy may represent a significant advance in the immunotherapy of human melanoma.

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Inhibition of Tumor-Induced Suppressor T Lymphocyte (Ts) Activity by Murine Interferon Beta (IFN-B). Deepak M. Sahasrabudhe, University of Rochester Cancer Center, Rochester, NY, 14642

In some tumor models inhibition of Ts-activity is a prerequisite to successful immunotherapy. Based on our data in the DNFB model (J Exp Med 166:1573, 1987) the effect of IFN-B on P815 mastocytoma-induced Ts-activity was evaluated.

In this model, concomitant antitumor immunity (Tc) peaks by Day 10 and is down regulated by Ts by Day 15. Cytotoxicity generated after a mixed lymphocyte tumor culture (MLTC) correlates with in vivo immunity and suppression of cytotoxicity correlates with in vivo Ts-activity.

Tumors were initiated by injecting 2 x 10^6 P815 cells subcutaneously on Day 1. IFN-B (10U, 1000U, 5000U) or buffer were injected i.v. every other day x 5 doses starting on Day 5. On Day 16, MLTC's were set up. Five days later a cytotoxicity assay was performed against ^{51}Cr labelled P815 cells. % specific lysis is shown. Numbers in parenthesis represent the dose of IFN-B.

E:T	Tc	Tc + Naive	Ts	Tc +Ts	Ts (10)	Tc +Ts (10)	Ts (1000)	Tc +Ts (1000)	Ts (5000)	Tc +Ts (5000)
50:1	88	81	0	19	6	22	23	20	81	84
25:1	84	76	0	12	2	21	1	21	63	75
12:1	78	79	2	15	3	24	6	23	58	81
6:1	70	69	1	7	0	9	0	20	38	64
3:1	56	55	0	8	1	13	0	12	23	48

Treatment with IFN-B 5000U every other day x 6 doses abrogated Ts-activity without adversely affecting cytotoxicity. IFN-B may be a useful adjunct in the immunotherapy of selected tumors.

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Anti-idiotype monoclonal antibody immunization therapy of cutaneous T cell lymphoma. Chatterjee, M., Foon, K., Seong, B.K., Barcos, M. and Kohler, H., Roswell Park Mem. Inst., Buffalo, NY 14263, and UCSD, San Diego, CA 92161.

Cutaneous T cell lymphoma (CTCL) is an indolent non-Hodgkin's lymphoma which is not cured by standard therapies once it reaches advanced stage. A novel approach to therapy is to use internal image anti-idiotype (Id) as an antigen (Ag) substitute for the induction of immunity. We have generated anti-Id mAb (Ab2) binding to a hybridoma SN2 (Ab1), which recognizes a unique glycoprotein, gp37, expressed by a subset of human leukemic T cells (J. Immunol. 139:1354, 1987). At least 2 of these Ab2 may indeed carry the internal image of the gp37 Ag (J. Immunol. 141:1398, 1988). Recently, we investigated the distribution of gp37 Ag by a sensitive immunoperoxidase staining method using mAb SN2. SN2 had a high specificity for T-leukemia/lymphoma cells and did not react with any normal adult tissues tested including thymus, lymphocytes, bone marrow cells, spleen, liver, kidney, lung, brain, heart, etc. CTCL cells from 5/11 out of 6 patients were strongly positive for gp37 Ag with intense surface membrane staining. The binding of radiolabeled SN2 to CTCL cells was studied for inhibition in the presence of the anti-Id mAbs 4EA2 and 4DC6 which mimic the gp37 Ag. Both clones inhibited the binding 100% and 80% respectively at a concentration of 50 ng. We also generated a murine Ab3 mAb (anti-anti-Id) by immunizing mice with the anti-Id mAb (Ab2). This Ab3 mAb reacts with CTCL cells in an identical fashion as the original Ab1 (SN2). Collectively, these data suggest that Ab2 4EA2 and 4DC6 may be useful for active immunotherapy of CTCL patients. We plan to study the CTCL patients in a phase I clinical trial to determine the effects of this type of therapy on various components of the immune system (both humoral and cellular) and try to identify the criteria to select patients who may benefit from anti-idiotype vaccine therapy.

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Syngeneic murine monoclonal antiidiotypes bearing the internal image of a human breast cancer associated antigen. J. Schmitz and H. Ozer. The Dept. of Microbiology, S.U.N.Y. at Buffalo, Buffalo, NY 14214 and the Division of Medical Oncology, The Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

According to Jerne's network theory, some antiidiotypes (Ab2) mimic external antigens recognized by specific antibodies (Ab1) and may be used in place of antigen for immunization. The murine monoclonal antibody F36/22 (IgG3, κ), specific for ductal carcinoma antigen (DCA) was used to generate syngeneic monoclonal antiidiotypes bearing the internal image of DCA. Female BALB/c mice were inoculated intraperitoneally every other week with 100 μg of F36/22 coupled to keyhole limpet hemocyanin; the first time in complete Freund's adjuvant and subsequently in incomplete adjuvant. Splenic lymphocytes were fused with the murine cell line P3X63 Ag8.653 3 days after the fourth immunization using 50% polyethylene glycol (PEG 4000). Two hybrids, MTO-1 and MTO-2, were selected based on the ability of culture supernatants to bind to F36/22 but not to the control antibody 2A31F6 (IgG3, κ) in an enzyme linked immunosorbent assay (ELISA) and cloned by limiting dilution. Paratope specificity of Ab2 was demonstrated in two ELISA assays. First, the binding of labeled F36/22 to DCA was inhibited 100% and 75% by 1.6 μg of MTO-2 and MTO-1 respectively. Second, the binding of labeled Ab2 to Ab1 was inhibited by purified DCA. MTO-1 neither enhances nor inhibits the binding of labeled MTO-2 to Ab1 although in the presence of MTO-2, binding of labeled MTO-1 is enhanced by 100% indicating that these Ab2 recognize distinct idiotopes. Rabbits immunized bi-weekly with MTO-1 or MTO-2 developed antibodies that bound specifically to DCA demonstrating that MTO-1 and MTO-2 bear the internal image of DCA. These data suggest that MTO-1 and MTO-2 could potentially be utilized to immunize high risk patients against progression or development of DCA positive tumors.